Jl S. Afr. Bot. 47 (3): 507–546 (1981)

POLYPLOIDY, CHROMOSOME INTERCHANGE AND LEAF SURFACE ANATOMY AS INDICATORS OF RELATIONSHIPS WITHIN *HAWORTHIA* SECTION *COARCTATAE* BAKER (LILIACEAE—ALOINEAE)

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Abstract

Original observations of the leaf surfaces and the number and morphology of the chromosomes were made on 282 plants of *Haworthia* section *Coarctatae* Baker from 26 localities in the Grahamstown/Port Alfred area of Cape Province, South Africa. Diploids ($2n = 14$), and all levels of polyploidy up to hexaploid were found in the area; also 14 chromosome interchanges and a small number of deletions and inversions were detected.

S.E.M. studies revealed that stomatal types can be characterised for *Haworthia coarctata* Haw. and *H. reinwardtii* Haw. sensu lato. Epidermal sculpturing is more frequently typical of individual populations, although some taxa were shown to be exceptionally variable within a population. A positive relationship was found between cell surface area and ploidy level.

The distribution of the interchanges gave no indication of inter-population relationships, but the observations on ploidy levels combined with those on anatomical features revealed that there are several relict diploid populations, from one of which polyploids have arisen to produce typical (i.e. tetraploid) *H. coarctata*. This has increased further in ploidy level to produce hexaploids of the same species and of *H. glauca* (S.D.) Bak.

Haworthia reinwardtii is normally diploid, but indications have been found of an eastward progression towards polyploids in the Chalumna area.

Uittreksel

POLIPLOÏEDIE, CHROMOSOOMWISSELING EN BLAAROPPERVLAKTE-ANATOMIE AS AANWYSERS VAN VERWANTSKAPPE IN *HAWORTHIA* SEKSIE *COARCTATAE* BAKER (LILIACEAE—ALOINEAE)

Oorspronklike waarnemings van die blaaroppervlaktes en die getal en morfologie van die chromosome is op 282 plante van *Haworthia* seksie *Coarctatae* Baker van 26 lokaliteite in die Grahamstad–Port Alfred-streek van die Kaapprovinsie, Suid-Afrika, gemaak. Diploïede ($2n = 14$) en alle vlakke van poliploïedie tot en met hexaploïed is in die streek gevind; ook 14 chromosoomwisselings en 'n klein getal segmentverliese en omkerings is ontdek.

A.E.M.-studies het aan die lig gebring dat stomatipes vir *Haworthia coarctata* Haw. en *H. reinwardtii* Haw. sensu lato gekarakteriseer kan word. Opperhuidgravering is meer dikwels tipies van individuele bevolkings, al is sommige taksa as uiters veranderlik binne 'n bevolking bewys. 'n Positiewe verwantskap is gevind tussen seloppervlaksarea en poliploïedievlak.

Accepted for publication 12th November, 1980.

Die verspreiding van die omkerings het geen aanwysing van tussenbevolkingsverwantskappe gegee nie, maar die waarnemings van ploïedie-vlakke saamgevat met die van anatomiese kenmerke het aangedui dat daar verskeie diploïede oorblyfselbevolkings is, en van een hiervan het poliploïede tot stand gekom en tipiese (d.w.s. tetraploïede) *H. coarctata* geproduseer. Die ploïede hiervan het verder toegeneem en hexaploïede van hierdie soort en van *H. glauca* (S.D.) Bak. geproduseer.

Haworthia reinwardtii is gewoonlik diploïed, maar aanduidings is gevind van 'n ooswaartse reeks na poliploïede in die Chalumna-streek.

INTRODUCTION

Little previous work has been reported on the anatomy of the leaf epidermis of *Haworthia* species. Cutler (1978a) showed that in *H. reinwardtii* f. *chalumnensis* differences in ploidy level and some chromosome interchanges could be detected by observation of the cuticular sculpturing, the shape of the outer cell walls and details of stomatal structure. He has also indicated the probable taxonomic significance of cuticular sculpturing in other *Haworthia* species (Cutler, 1979), and there is now extensive evidence that sculpturing and stomatal structure are under rigid genetical control and can be used as very precise guides to the identity of taxa within the *Aloineae* (Brandham and Cutler, 1978; Cutler and Brandham, 1977).

It has already been shown that *H. reinwardtii* f. *chalumnensis* is extremely unstable with regard to its chromosome complement, in which interchange hybridity is widespread (Brandham, 1974). This high frequency is unique in the *Aloineae*, more interchanges having been found in f. *chalumnensis* than in the rest of the *Aloineae* put together. We therefore decided to look to the west of the area where f. *chalumnensis* grows and investigate other populations of the group to which the taxon belongs (the section *Coarctatae* Baker of *Haworthia*) with the object of determining: a) the frequency and distribution of chromosome interchanges and other structural chromosome mutations, and b) whether any which were found had an effect on the morphology of the leaf surface.

Bayer (1976) has reviewed the taxonomy of *Haworthia*, and his findings with respect to *H. reinwardtii, H. coarctata*, their many varieties and forms and related species are particularly interesting. In his attempts to rationalise a complex problem he suggested a geographical and morphological basis for the recognition of *H. reinwardtii* and *H. coarctata* (Bayer, 1973).

Initial observations on stomatal details of plants collected by ourselves and/or Bayer from the Eastern Cape Province, indicated on the map (Fig. 1), showed some puzzling discontinuities when considered with Bayer's revised names for the many forms of *H. reinwardtii* and *H. coarctata* described from that area. A combined cytological and anatomical approach seemed appropriate to try to interpret the significance of these anomalies and to see whether a greater understanding of the complex interrelationships of the plants concerned could be achieved.

In addition, a marked variation in epidermal cell and stomatal size was found to exist between plants from different localities, and an investigation was carried out to determine whether this variation was correlated with differences of ploidy level.

MATERIAL AND METHODS

Material

Living material was collected from 282 plants growing in 26 localities in the general area of Grahamstown, Cape Province by Bayer or by Bayer, Brandham and Cutler (Table 1, Fig. 1). Plants of *Haworthia* sect. *Coarctatae* form extensive clumps of prostrate branches rooting along their length, and it was necessary to collect only the tip of one branch from each for cultivation and subsequent anatomical and cytological study. Thus unnecessary damage to the populations of these uncommon succulents was avoided. Plants were cultivated at Kew and representative vouchers are deposited in the Kew Herbarium (K).

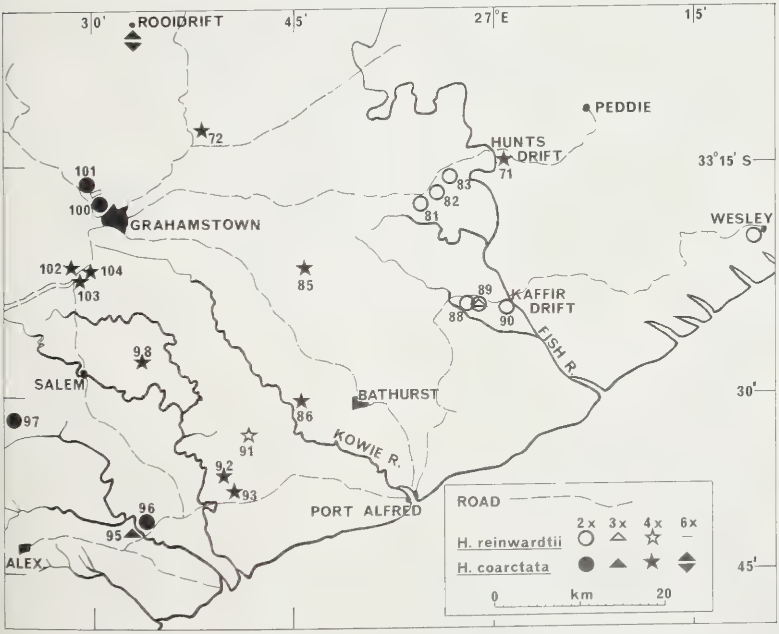


FIG. 1.
Map of the Grahamstown–Port Alfred area showing the location of the majority of the sampled sites of *H. coarctata* and *H. reinwardtii* (see Table 1), also the ploidy level of the plants at each site.

TABLE 1.
Origin of material, chromosome number and distribution of chromosome mutations.

Species	*Loc. no.*	*Locality*	*2n*	*No. of plants studied*	*Chr. aberration and frequency*	*Fig.*
Haworthia coarctata Haw.	71	Hunt's Drift	28	11	None	—
H. coarctata subsp. *adelaidensis* (von Poelln.) Bayer	72	Queens Road, Ecca Pass 13 km N E Grahamstown	28	11	2 with INT. 1 4 with INT. 2	4a 3f, 4d
H. reinwardtii Haw. var. *brevicula* Smith	81	Fraser's Camp, Near Fort	14	27	None	—
H. reinwardtii Haw. var. *brevicula* Smith	82	Fraser's Camp, 1 km E Fort	14	4	None	—
H. reinwardtii Haw. var. *huntsdriftensis* Smith	83	3 km E Fraser's Camp Fort	14	10	None	—
H. coarctata Haw.	85	Blaauw Krantz Rly. Bridge, Martindale-Grahamstown Rd	28	13	None	—
H. coarctata Haw.	86	Kowie River, S of White Krantz, 32 km S E Grahamstown	28	20	None	—
H. reinwardtii Haw. f. *kaffirdriftensis* (Smith) Bayer	88	Near Causeway on Cap River, W of Kaffir Drift	14	6	1 with INT. 12	3a, 4m
H. reinwardtii Haw. f. *kaffirdriftensis* (Smith) Bayer	89	½ km E of 88	14 21	5 4	None None	— —
H. reinwardtii Haw. f. *zebrina* (Smith) Bayer f. *olivacea* (Smith) Bayer and intermediates	90	Kaffir Drift	14	26	1 with INT. 5	3c, 4g

Species	*Loc. no.*	*Locality*	*2n*	*No. of plants studied*	*Chr. aberration and frequency*	*Fig.*
H. reinwardtii Haw. (approaching *H. coarctata* morphologically)	91	Between Port Alfred and Southwell	28	17	2 with INT. 1A 1 with INTS. 1A and 4 3 with INT. 3 1 with INT. 3 and DEL. 2 1 with INTS. 3 and 4A 2 with DEL. 1	4b 2a, 4c 2c 4e 2b, 4f 4o
H. coarctata Haw.	92	Hopewell Farm, 41 km S Grahamstown on Southwell to Kenton Rd.	28	19	1 with INT. 6 1 with INT. 6 and 7	— 3b, 4h
H. coarctata Haw.	93	½ km S E of 92	28	7	All with INT. 8	4i
H. coarctata subsp. *coarctata* var. *tenuis* (Smith) Bayer	95	Harvest Vale, 12 km E Alexandria	21	6	None	—
H. coarctata subsp. *coarctata* var. *tenuis* (Smith) Bayer	96	½ km E of 95	14	8	2 with INT. 9	2d, 4j
H. coarctata Haw. (variable)	97	Salem Heights, 12 km S Salem	14	18	None	—
H. coarctata Haw.	98	9½ km W Vaalvley on Salem Rd.	28	7	5 with INV. 1	4n
H. coarctata subsp. *adelaidensis* (von Poelln.) Bayer	100	2 km N Grahamstown on Cradock Road	14	11	None	—

Species	*Loc. no.*	*Locality*	*2n*	*No. of plants studied*	*Chr. aberration and frequency*	*Fig.*
H. coarctata subsp. *adelaidensis* (von Poelln.) Bayer	101	6 km N Grahamstown, north of airfield	14	10	None	—
H. coarctata subsp. *coarctata* var. *greenii* (Baker) Bayer	102	Howiesonspoort. Ridge opposite Nature Reserve	28	5	None	—
H. coarctata subsp. *coarctata* var. *greenii* (Baker) Bayer	103	Howiesonspoort. At Bridge	28	13	1 with INT. 10 1 with INT. 11 1 with DEL. 3	3d, 4k 3e, 4l 4p
H. coarctata subsp. *coarctata* var. *greenii* (Baker) Bayer	104	Howiesonspoort. River cliff	28	2	1 with DEL. 3	4p
H. reinwardtii aff. f. *chalumnensis* (Smith) Bayer	Bayer 91	Wesley	14	3	None	—
H. reinwardtii f. *chalumnensis* (Smith) Bayer	Bayer 85–90	Chalumna area	21 28	47 98	Many. See Brandham (1974), Cutler (1978a)	—
H. coarctata Haw.	Bayer s.n.	Rooidrift	42	1	None	—
H. glauca Bak.	117	Between Uitenhage and Steytlerville	42	10	1 with $2n = 40$ 1 with $2n = 43$	—
H. glauca var. *herrei* f. *armstrongii* (von Poelln.) Bayer	112	9 km N E of Uitenhage	35	8	None	—

Cytological Methods

Somatic chromosomes were prepared from actively elongating root tips which were collected in the summer months when plants were growing the most quickly. Optimum root growth was found about 2–3 days after the plants were watered. Root tips were pretreated in saturated aqueous alpha-bromonaphthalene for 24 hours at 4 °C, fixed in 1:3 acetic alcohol, hydrolysed in normal hydrochloric acid at 60 °C for 7 minutes and stained in Feulgen using a standard technique. Squash preparations of the stained root tips were made permanent, using the liquid CO_2 method of Bowen (1956) and are kept in the collection of the Cytogenetics section of the Jodrell Laboratory as reference material. Photographs of chromosomes were taken on a Zeiss photomicroscope and drawings were made with the aid of a Wild drawing attachment at an original magnification of ×3000

Anatomical Methods

The methods of preparation for scanning electron microscope (S.E.M.) studies were as in Cutler (1979). Photographs were taken in a JEOL JSM 35 instrument at 18 KeV at a constant tilt of 30°. The leaf surfaces were photographed with the long axis either vertical or horizontal with respect to the page. Prints were selected from these to show the relief to the best advantage. Selected photographs are reproduced here mostly at a magnification of c. ×300 with a small number at c. ×40 or ×660.

For light microscope studies FAA-fixed material was washed and sectioned at c. 25 μm, stained in safranin and haematoxylin as in Cutler (1978b) and permanent slides were prepared.

Cell sizes were compared by counting the number of cells present in a standard area. Allowances were made when more than one stoma were present in the standard area.

CYTOLOGICAL RESULTS

Karyotype Structure and Chromosome Number

The basic karyotype (i.e. excluding structural chromosome mutations such as interchange) found in all plants was typical of that which occurs throughout the *Aloineae*, a tribe which is almost unique in the plant kingdom in the uniformity of its chromosome pattern. The karyotype is markedly bimodal, with diploids having $2n = 14$ chromosomes. Four pairs are long and three pairs are much shorter, about a third of the length of the long ones. The short chromosomes are difficult to distinguish from each other and no attempt will be made to do so here. They are all acrocentrics, and have a characteristic constriction in the mid-point of the long arm. This is not a secondary constriction in the generally-accepted sense (i.e. nucleolar-organising), but is simply a narrower segment which could be heterochromatic, since it can be enhanced by cold treatment (Riley and Majumdar, 1979). Differential staining of *Aloineae* chromosomes has been achieved by Ghosh and Roy (1977), using giemsa, but we have not been able to repeat their results.

The four pairs of long chromosomes can also be classified as acrocentrics, although the short arms of some are quite substantial. They are identified as follows: The L_1 has the longest short arm and is always readily recognised. The L_2 is the longest overall and has a constriction in the short arm similar to that in the long arm of the short chromosomes. The L_3 and L_4 are somewhat similar to each other, but the L_4 is shorter and usually has the shortest short arm.

In the *Aloineae* as a whole the most frequent level of ploidy is the diploid (Brandham, 1971). Nevertheless, polyploids are common in some sections of *Haworthia*, in particular that section which is the subject of this investigation, the *Coarctatae* (Brandham, 1971, 1974). The chromosome number of every plant examined here is given in Table 1, which also includes a summary of the findings of our earlier work on triploid and tetraploid *Haworthia reinwardtii* f. *chalumnensis* (Brandham, 1974; Cutler, 1978a).

Of the 26 localities that were sampled 16 contained *H. coarctata*, eight contained *H. reinwardtii* and two, *H. glauca*. Of the eight populations of *H. reinwardtii* seven were diploid ($2n = 14$), although one of these also contained triploids ($2n = 21$). Triploid individuals have been recorded previously as occurring sporadically in diploid populations of several other members of the *Aloineae*. These are usually autotriploids, and are formed directly by diploids as a result of the fusion of a non-reduced gamete with a normal one (Brandham, 1971; Abraham and Prasad, 1979).

Only one of the eight populations of *H. reinwardtii* was tetraploid ($2n = 28$), and interestingly this population (from locality 91, see Fig. 1) approached *H. coarctata* morphologically (Bayer, pers. comm.). Farther east, *H. reinwardtii* becomes exclusively polyploid, the variety *peddiensis* Smith being triploid and the forma *chalumnensis* being triploid or tetraploid in all its known localities east of Wesley (Brandham, 1974).

Polyploidy is much more common in *H. coarctata*, with plants from 10 of the 16 sampled localities being tetraploid, and a single plant from one locality, Rooidrift, being hexaploid ($2n = 42$). Four of the 16 localities contained diploid plants of this species (localities 96, 97, 100, 101) and locality 95 apparently contained only triploids.

Triploids either are sterile or they produce other than eu-triploid seed progeny when selfed or crossed with each other, the possible range of chromosome number extending from diploid to tetraploid via a large number of aneuploids (Johnsson, 1942). As there is no deviation from triploidy at locality 95 it is quite probable that the population is composed entirely of a single clone spreading vegetatively, although some anatomical variability between individuals of this population was noted (see below). Other evidence establishes the existence of two other clonal populations among the 26 populations studied (localities 93 and 112, see below).

Of the two sampled populations of *H. glauca* one comprised mostly hexaploid individuals with $2n = 42$ chromosomes (locality 117), but two of the ten individuals examined were aneuploids with $2n = 40$ or 43. Aneuploidy at this frequency is to be expected in populations of sexually-reproducing high level autopolyploids.

The other population of this species, *H. glauca* var. *herrei* f. *armstrongii* (syn. *H. armstrongii* von Poelln.) was collected from its type locality (no. 112), which is the only station where the taxon is known to occur (Bayer, 1976). All sampled plants were pentaploid ($2n = 35$), which again indicates that the population is a single clone. Bayer (1976) suggested that the taxon could be of hybrid origin, and this chromosome number confirms the idea.

Pentaploidy can arise naturally by any one of a number of routes. The most obvious of these are tetraploid × hexaploid hybridisation, or (much less likely in *Haworthia*) octoploid × diploid, but there are others which involve the fusion of normal gametes with non-reduced ones (i.e. those having the same chromosome number as that of the plant in which they arise, a result of meiotic failure). Pentaploids produced in this way can arise from crosses between tetraploid and non-reduced triploid or between diploid and non-reduced tetraploid.

All of the four pathways given here for the formation of pentaploids involve the crossing of plants with different ploidy levels, so it is very probable that the majority of naturally-occurring pentaploids are allopentaploids of hybrid origin. As far as *H. glauca* var. *herrei* f. *armstrongii* is concerned the present extent of our knowledge of the morphology and ploidy levels of nearby populations of *Haworthia* spp. is insufficient to allow determination of the precise parentage of this taxon, although it will be noted below that it has close affinities with tetraploid *H. coarctata*.

Chromosome Mutations

Fourteen chromosome interchanges have been found in the *Coarctatae* studied in this investigation, also at least one deletion (possibly three), and one pericentric inversion. In the diploids all mutations were heterozygous, and in tetraploids they were in the simplex heterozygous condition, i.e. having only a single morphologically altered chromosome in the case of deletions and inversions and only two altered chromosomes in each interchange. This is in common with the great majority of reported cases of chromosome interchange, both in *Haworthia* and in the rest of the *Aloineae* (Riley, 1968; Riley *et al.*, 1967; Riley and Majumdar, 1968; Brandham, 1974).

Full homozygosity for interchanges has never been found in wild-collected plants of any taxon in the *Aloineae*, and the duplex heterozygote in tetraploids (i.e. with two pairs of altered chromosomes per interchange) has been observed in only a very few individuals in a single population of *H. reinwardtii* f. *chalumnensis* (Brandham, 1974). There is also one record of a duplex deletion heterozygote in tetraploid *Aloe elgonica* (Brandham and Johnson, 1977a).

The frequency and distribution of the various mutations found in the present study are given in Table 1 and a description of each one follows. They are numbered in the order in which they were discovered. All occurred either in diploids or tetraploids, no structural chromosome mutation being found among the triploids, pentaploids or hexaploids.

Interchange 1: in *H. coarctata* subsp. *adelaidensis* (4x), loc.72. Breaks have occurred in the long arms of an L_1 and an L_3 chromosome and distal segments of unequal lengths have been exchanged to give a longer L_1 and a shorter L_3 (Fig. 4a).

Interchange 1A: in *H. reinwardtii* (4x), loc.91. This interchange is almost identical to interchange 1, with again a longer L_1 and a shorter L_3 resulting. Nevertheless interchanges 1 and 1A cannot be the same mutation because they occur in different taxa growing a considerable distance apart. The difference was confirmed by measurement of the long arms of the interchange chromosomes. In interchange 1 the shorter long arm is less than half the length of the longer, the mean ratio of length being 1:2.13, and in interchange 1A it is more than half (mean ratio 1:1.86). Interchange 1A was found alone in two individuals (Fig. 4b) but was associated with interchange 4 in a third individual from the same locality (Figs 2a, 4c).

Interchange 2: in *H. coarctata* subsp. *adelaidensis* (4x), loc.72. This is a centric fusion type of interchange with breaks at the centromere of an L_1 chromosome and one of the short chromosomes. The short arm of the L_1 and the long arm of the short chromosome have reunited to produce a metacentric which is somewhat longer than the normal short chromosomes. The long arm of the L_1 and the short arm of the short chromosome have united to produce a long chromosome which is difficult to distinguish from the normal L_3 or L_4 chromosomes (Figs 3f, 4d).

Interchange 3: in *H. reinwardtii* (4x), loc. 91. Here breaks have occurred near the centromeres of an L_3 and an L_4 chromosome with reunion of the long arms producing a very long metacentric and of the short arms, producing a short acrocentric (Fig. 2c). Because the longer arm of the short interchange chromosome is longer than the short arms of either the L_3 or the L_4 it is evident that it must also contain the proximal segment of a long arm. It is therefore apparent that the breaks involved in this interchange are not through the centromere but are proximal in the long arm of the L_3 and the short arm of the L_4 respectively. At locality 91 interchange 3 occurs either alone (Fig. 2c) or with deletion 2 (Fig. 4e) or with interchange 4A (Figs 2b, 4f).

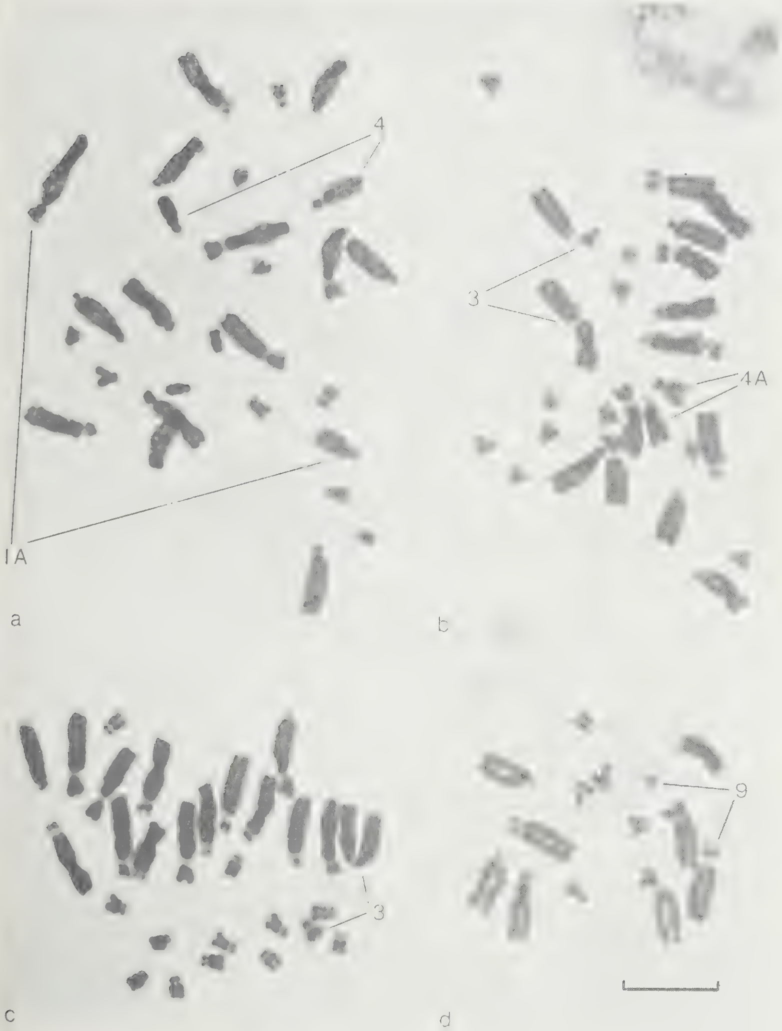


FIG. 2.
Somatic chromosomes of interchange heterozygotes. Scale = 10 μm. a: INTS. 1A and 4 (loc. 91). b: INTS. 3 and 4A (loc. 91). c: INT. 3 (loc. 91). d: INT. 9 (loc. 96). The identity of the plants at localities numbered in Figs 2, 3 and 4 is given in Table 1.

Interchange 4: in *H. reinwardtii* (4x), loc. 91. Breaks have occurred in the long arms of an L_4 and a short chromosome, with reunion to produce two chromosomes of intermediate and almost equal length. This exchange was found in a single plant which also contained interchange 1A (Figs 2a, 4c).

Interchange 4A: in *H. reinwardtii* (4x), loc. 91. This is similar to interchange 4, but with an L_2 and a short chromosome being involved. The two interchange chromosomes are again of intermediate length, but one is considerably longer than the other (Figs 2b, 4f). Interchange 4A was also found in a single plant only, being associated with interchange 3.

Interchange 5: in *H. reinwardtii* (2x), loc. 90. This interchange involves an L_2 and an L_4 chromosome and results in the production of a very long metacentric and a very short one (Figs 3c, 4g). Normal L_2 chromosomes have a constriction (not a nucleolar-organiser) in the short arm. This constriction can be seen in the long metacentric in some favourable cells, indicating as in interchange 3 that the break points are not through the centromeres but are located proximally in the short arm of the L_2 and the long arm of the L_4.

Interchange 6: in *H. coarctata* (4x), loc. 92. This results from breaks in the short arm of an L_1 and near the distal end of the long arm of an L_2 with reunion to produce a longer L_1 short arm and a shorter L_2 long arm (Figs 3b, 4h). This interchange was found alone in one plant and associated with interchange 7 in another.

Interchange 7: in *H. coarctata* (4x), loc. 92. Two short chromosomes are involved in this exchange. Breaks have occurred at the centromere with reunion to produce a very short metacentric derived from the two short arms and a metacentric derived from the long arms. This is a little longer than a normal short chromosome (Figs 3b, 4h). This interchange was found in only one plant, which also carried interchange 6.

Interchange 8: in *H. coarctata* (4x), loc. 93. A large segment of an L_2 long arm has been exchanged for the distal portion of the long arm of a short chromosome to produce a short interchange chromosome a little longer than a normal short chromosome and a longer interchange chromosome similar in length to many long chromosomes. The latter carries a proximal constriction on the long arm which is the equivalent of the constriction often seen in the long arm of the short chomosomes, and enables the break points to be determined precisely (Fig. 4i). Every plant sampled from locality 93 was a simplex heterozygote for this mutation, indicating either that this situation is positively selected in seed production or that the entire population is a single clone. Although the former alternative cannot be excluded until confirmatory breeding experiments are carried out, it would seem that the latter (clonal) alternative is the more likely, especially when it is recalled that a) this entire group of *Haworthia* is capable of spreading vegetatively by means of creeping and branching stems, and b) it has

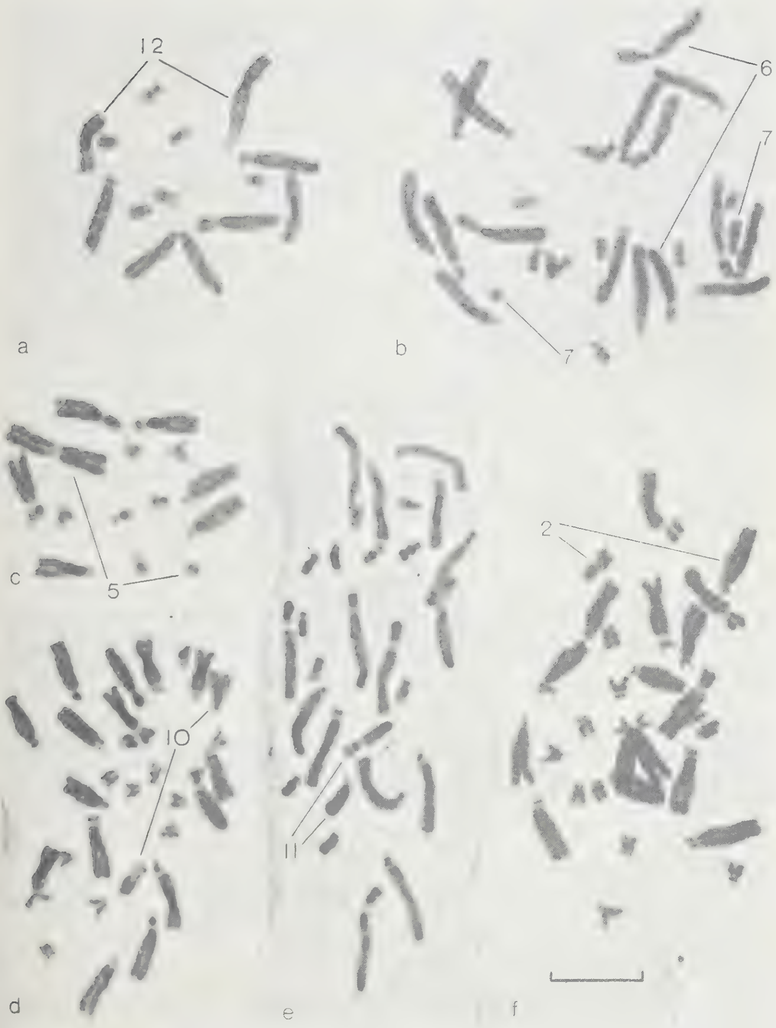
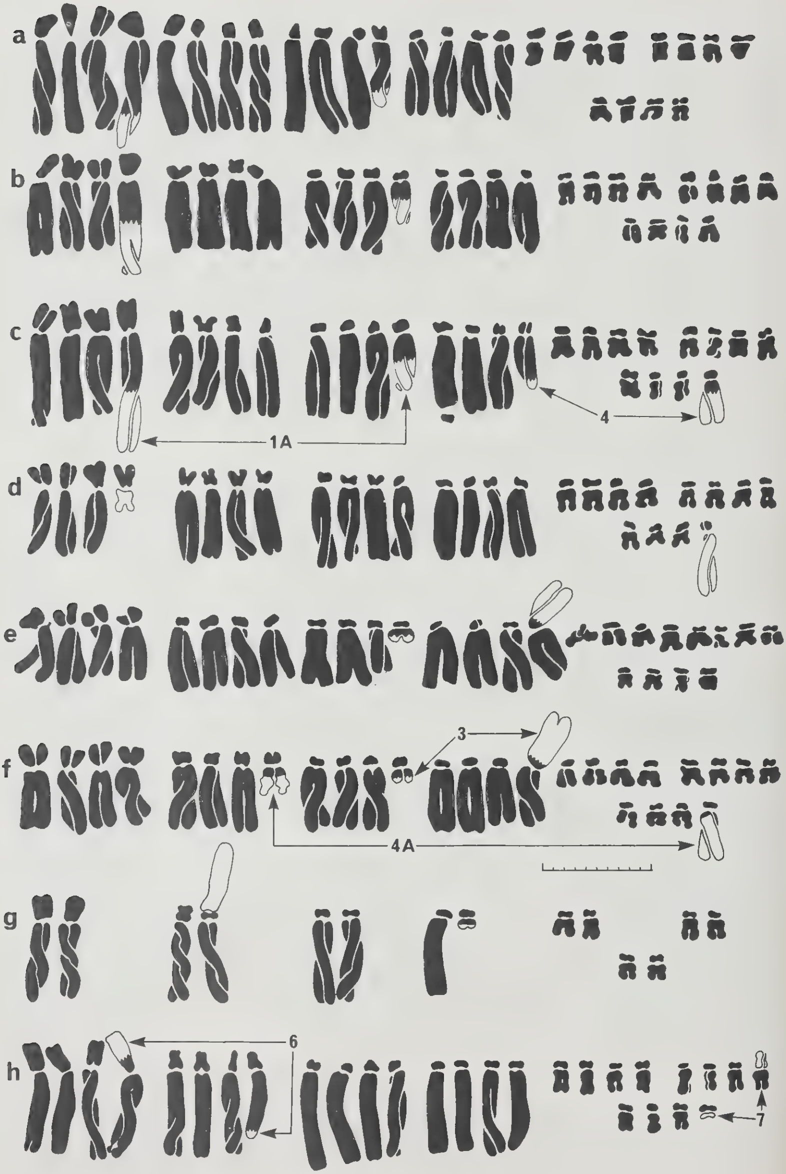


FIG. 3.
Somatic chromosomes of interchange heterozygotes. Scale = 10 μm. a: INT. 12 (loc. 88). b: INTS. 6 and 7 (loc. 92). c: INT. 5 (loc. 90). d: INT. 10 (loc. 103). e: INT. 11 (loc. 103). f: INT. 2 (loc. 72).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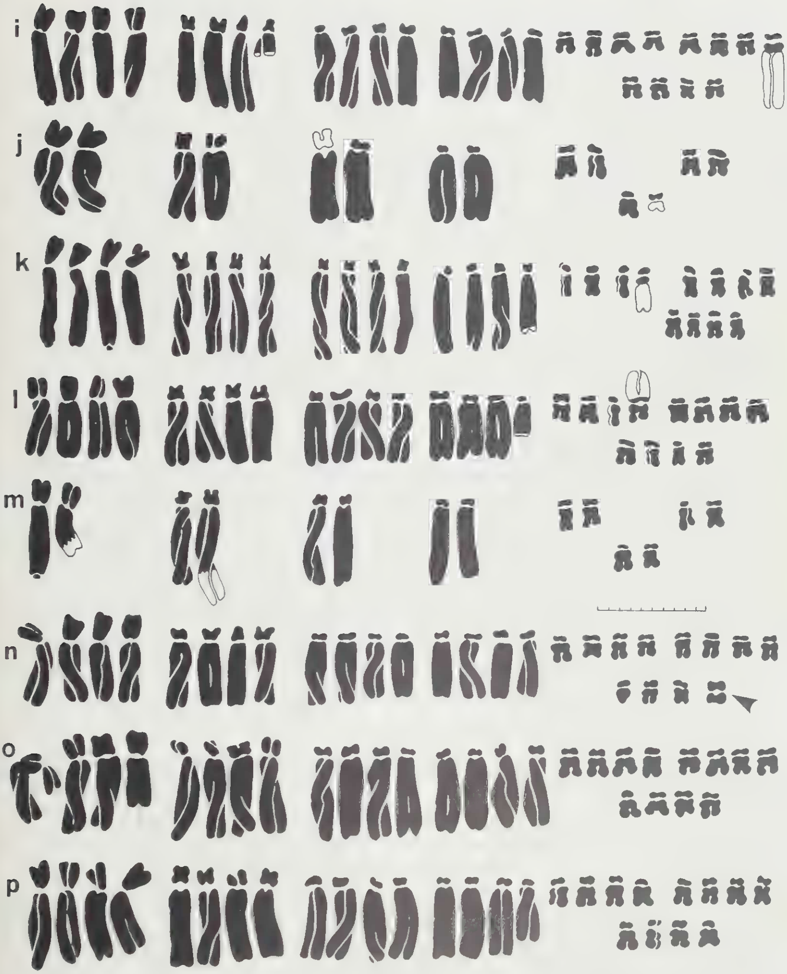

FIG. 4.

Diploid and tetraploid karyotypes showing 18 structural chromosome mutations found in *Haworthia* sect. *Coarctatae*. Interchanged chromosome segments are left unshaded for clarity. The boundary between each one and the rest of the chromosome is straight if the position of the break point is known and irregular if not precisely known. Deletions are shown by the missing portions being dotted. Scale = 10 μm. a: INT. 1 (loc. 72). b: INT. 1A (loc. 91). c: INTS. 1A and 4 (loc. 91). d: INT. 2 (loc. 72). e: INT. 3 and DEL. 2 (loc. 91). f: INTS. 3 and 4A (loc. 91). g: INT. 5 (loc. 90). h: INTS. 6 and 7 (loc. 92). i: INT. 8 (loc. 93). j: INT. 9 (loc. 96). k: INT. 10 (loc. 103). l: INT. 11 (loc 103). m: INT. 12 (loc. 88). n: INV. 1, a pericentric inversion giving a centromere shift (arrow) on a short chromosome (loc. 98). o: DEL. 1 (loc. 91). p: DEL. 3 (loc. 103). The identity of the plants at each locality is given in Table 1.

already been shown that another population of *H. coarctata* (at loc. 94) is also probably a clone, all specimens sampled being triploid.

Interchange 9: in *H. coarctata* subsp. *coarctata* var. *tenuis* (2x), loc. 96. This is a case of the breakage at the centromeres of an L_2 and a short chromosome. The two short arms have reunited to produce a very short submetacentric and the two long arms have joined to produce a long chromosome which is a little longer than normal. It resembles the L_1 chromosomes but can be distinguished from them by the constriction in the mid-point of its short arm, which is characteristic of the long arm of most short chromosomes (Figs 2d, 4j).

Interchange 10: in *H. coarctata* subsp. *coarctata* var. *greenii* (4x), loc. 103. Breaks have occurred at the constriction in the long arm of a short chromosome and at about three-quarters of the distance from the centromere to the end of the long arm of an L_4 chromosome. Exchange of segments gives two interchange chromosomes of intermediate length (Figs 3d, 4k).

Interchange 11: in *H. coarctata* subsp. *coarctata* var. *greenii* (4x), loc. 103. In this exchange a large portion of the long arm of an L_4 chromosome has been transferred onto the short arm of a short chromosome (Fig. 3e). It is not possible for the transferred fragment to join onto the telomere of the short chromosome's short arm, because the latter is a stable "healed" structure with which other chromosome material cannot normally fuse. Therefore there must have been a break either within that short arm or through the centromere. It is significant that in this exchange the break in the L_4 chromosome is patently not through the centromere and it has been pointed out (Brandham, 1974) that in a chromosome interchange if one break is not at the centromere the other also cannot be through the centromere. Thus the break in the short chromosome is interstitial in the short arm, even though this region is very short indeed, and the interchange has been drawn in this way in Fig. 4l.

Interchange 12: in *H. reinwardtii* f. *kaffirdriftensis* (2x), loc. 88. This is a simple exchange of different-sized portions of the long arms of an L_1 and an L_2 chromosome and results in a shorter L_1 and a longer L_2 (Figs 3a, 4m).

Inversion 1: in *H. coarctata* (4x), loc. 98. In five plants out of seven sampled from the site one of the short chromosomes was metacentric, although its length was unchanged. The remaining 27 chromosomes were apparently normal, so the plants are provisionally considered to be simplex heterozygotes for an asymmetrical pericentric inversion which has resulted in a shift in the position of the centromere (Fig. 4n). The existence of this inversion cannot be proved until meiotic and/or breeding studies confirm whether or not the characteristic phenomena associated with such inversions occur, in particular the formation of the duplicate/deletion chromosomes which are visible at AI or MII or meiosis in these plants and which sometimes survive in their progeny (Brandham and Johnson, 1977b).

Deletion 1: in *H. reinwardtii* (4x), loc. 91. In two plants sampled from this locality one of the L_1 chromosomes was considerably shortened. No other chromosome was correspondingly longer than normal, so a balanced interchange can be ruled out. In the absence of other evidence it seems that a deletion has occurred, with the loss of the distal portion (Fig. 4o). Deletions of this type are probably lethal in diploids, but have been recorded previously in triploid and tetraploid *Aloineae*, occurring both in natural populations (Brandham and Johnson, 1977a) and in the cultivated progeny of normal plants (Brandham and Cutler, 1978).

Deletion 2: in *H. reinwardtii* (4x), loc. 91. At this locality one plant contained interchange 3, and additionally an L_3 chromosome which was somewhat shorter than normal (Fig. 4e). This is apparently another simple deletion since there is not a fourth abnormal chromosome in the plant to compensate for the loss. However, reference to Table 1 will reveal that this population also contains interchange 1A (Figs 4b, 4c) in which the shorter interchange chromosome is an apparently shortened L_3. Thus it is quite possible that the "Int 3, Del 2" plant contains interchange 3 and one only of the two chromosomes of interchange 1A, inherited as an unbalanced, though viable, gamete from one of its parents.

Deletion 3: in *H. coarctata* subsp. *coarctata* var. *greenii* (4x), loc. 103. As in the previous case this plant has a single chromosome shorter than normal, an L_4 (Fig. 4p), but again it should be noted from Table 1 that the population also contains interchange 10 (Fig. 4k), in which one of the affected chromosomes resembles a shortened L_4. It is thus quite likely that the deletion heterozygote figured in Fig. 4p is another unbalanced interchange heterozygote carrying only one of the two altered chromosomes of interchange 10, the other being replaced by a normal one.

ANATOMICAL RESULTS

Leaf Surface Anatomy

The leaf surface anatomy typical of the complexes *H. reinwardtii* and *H. coarctata* will be described first. These will be followed by notes on the surface features of plants from all localities indicated in Figure 1 and Table 1. Selected photographs show the range of variability which occurs, and specific examples of plants of significance. Figure 5 shows cross-sections of stomata of a number of plants. It illustrates the relationship of lobes, subsidiary cells and guard cells and assists with the interpretation of the SEM photographs.

(a) The "*H. reinwardtii*" type (Figs 5a, 5c, 6)

Epidermal cells are 4-5-6-7 sided, normally slightly longer than wide. The position of the anticlinal walls is indicated by shallow, narrow grooves. Epidermal cell outer walls are convex to a greater or lesser degree and in some examples from the eastern end of the range of the species they are produced

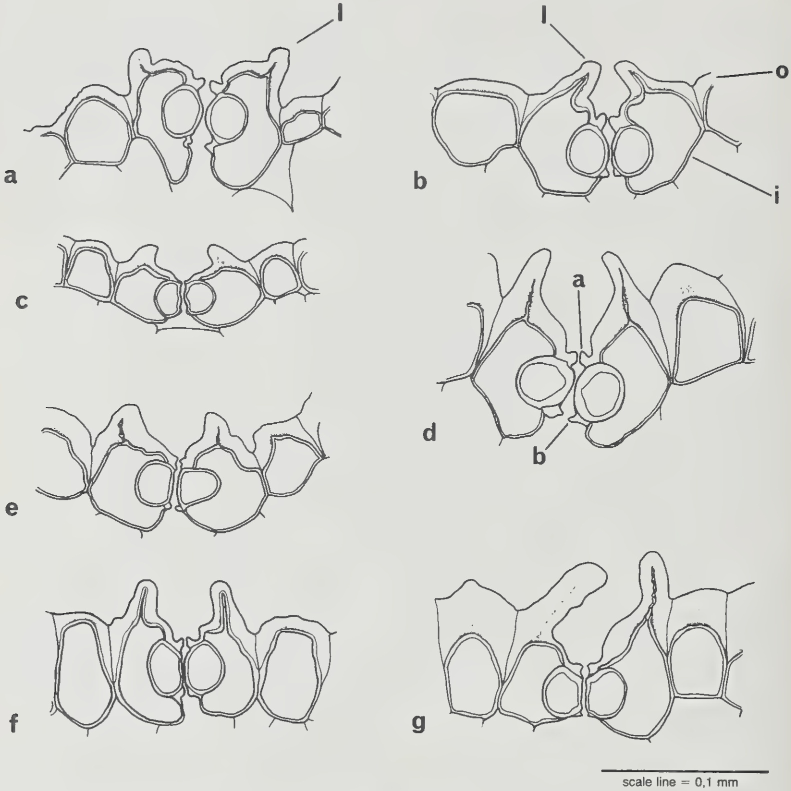

Fig. 5.

Transverse sections through stomata and adjacent epidermal cells. Scale = 100 μm. a: *H. reinwardtii* forma *chalumnensis* (73–1007, tetraploid). Note position of lateral lobes of rim to suprastomatal cavity (l). b: *H. coarctata* (loc. 98, tetraploid.) Note overarching lateral lobes of rim to suprastomatal cavity. c: *H. reinwardtii* var. *huntsdriftensis* (loc. 83, diploid). Similar to a above but its cells are smaller. d: *H. glauca* var. *herrei* forma *armstrongii* (loc. 112–1279, pentaploid). This plant has a stomatal structure similar to that of *H. coarctata* in b above, but all cells are larger. e: *H. coarctata* subsp. *coarctata* var. *tenuis* (loc. 96, diploid). This plant has stomata reminiscent of *H. reinwardtii s.l.*. f: *H. coarctata* subsp. *coarctata* var. *tenuis* (loc. 95, triploid). This plant shows stomata with a tendency towards the type found in *H. coarctata*. g: *H. glauca* (loc. 117–1325, hexaploid). This plant has long and irregular lobes surrounding the suprastomatal cavity but is otherwise like *H. coarctata.*

In the diagrammatic representation of the sections above, at o, the outer part of the epidermal cell wall and cuticle are not drawn separately. The cuticle is relatively thin, but the outer wall is extensively cutinized. Some granular inclusions are shown but the clearly stratified nature of this outer part of the wall is not indicated, for the sake of clarity. The thinner, inner part of the cell wall, i, is not impregnated with cutin and takes up safranin in the preparations, whereas the outer part of the wall remains clear. The stomata have outer (a) and inner (b) cuticular ledges developed to a greater or lesser degree.

into a conical, more or less central papilla. A sculpturing of micropapillae is evident. This is composed of cell wall and cuticle. *Stomata* are deeply sunken. The outer cuticular rim is visible from above, but the regulated aperture is difficult to see except in T.S. The most prominent feature is the raised rim composed of four lobes, one from each of the cells adjacent to the guard cells (subsidiary cells). The lobes are ridges of outer wall, accentuated by cuticle, arising along the long axis of flanking cells and across the width of the polar cells. On the flanking cells the ridge arises along the centre of the wall; away from the stoma the outer part of the wall bears micropapillae which are normally larger than those on other epidermal cells, but towards the guard cells the outer wall is more or less smooth and slopes gently inwards. On the polar cells the lobes arise from the outer wall along the side nearest to the guard cells. The outer aperture defined by the lobes is rectangular with the long axis parallel to the long axis of the leaf. The lobes are upright to slightly overarching.

Wax is present as a continuous thin sheet and as sparse crystals in the form of flakes or as larger irregular particle

(b) The "*H. coarctata*" type (e.g. Figs 5b, 5e, 5f, 7)

Very similar in many respects to the "*H. reinwardtii*" type but *epidermal cells* exhibit a wider range of sculpturing, and in many specimens the micropapillae are more pronounced. Larger central papillae are a common feature. Anticlinal wall position is sometimes related to narrow bands of unsculptured surface around cells. The most conspicuous differences relate to the *stomata*. The guard cells are deeply sunken, and the outer cuticular rim can normally be seen only with difficulty from above because the thick lobes forming the rim are normally strongly overarching. The lobes on the flanking cells are set towards the edge of the cell nearest the guard cell pair. This together with the fact that the part of the outer wall nearest to the guard cells slopes steeply inwards gives a characteristic appearance to the stomatal apparatus.

Wax is as in the "*H. reinwardtii*" type

(c) Individual descriptions of plants from each locality

The main differences of significance relate to stomatal structure, principally the subsidiary cell lobing. The individuals described here are selected from the range of material studied from each locality and are representative of that locality. Where the individuals differ widely at a particular locality, the range of variation is described. This is most marked where there are differences of ploidy level. Descriptions of epidermal cell structure refer to outer walls only.

It may be assumed that micropapillae on subsidiary cells are coarser than those on normal epidermal cells, unless otherwise stated.

Loc. 71: *H. coarctata*, 4x (Fig. 7a): *Epidermal cells* with strongly domed outer walls; cuticular sculpturing composed of micropapillae showing some tendency towards aggregation into irregular ridges radiating from centre of cell. *Stomata* with very strongly overarching lobes, outer pore small, with square outline.

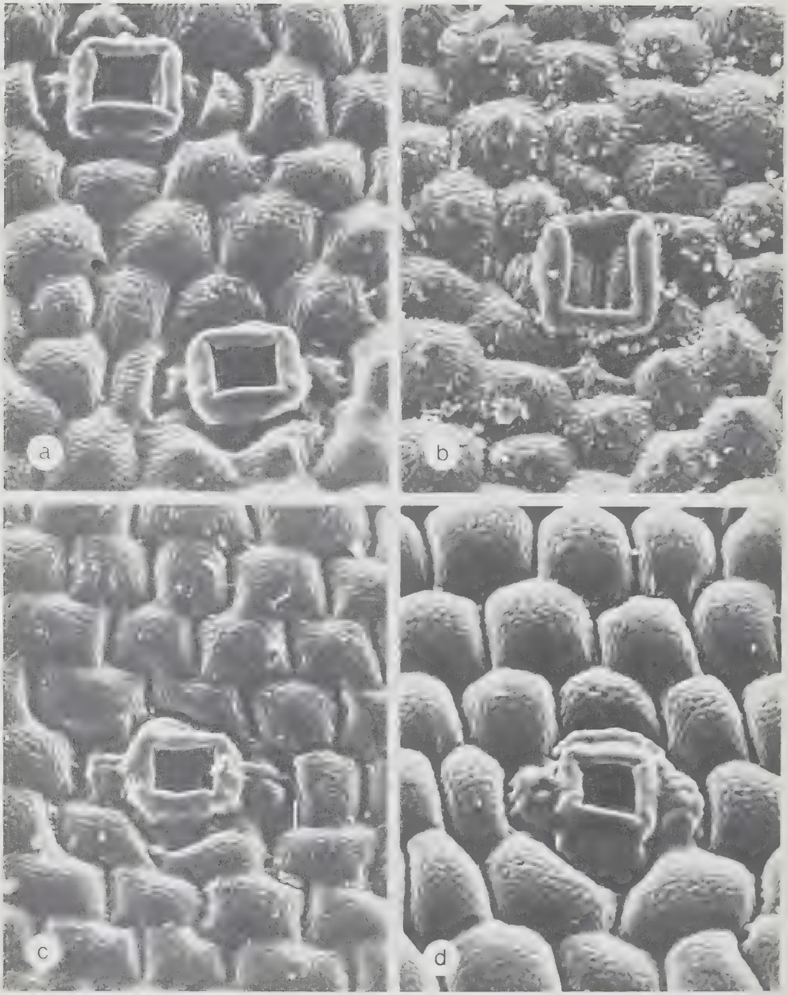

FIG. 6.
Leaf epidermis of *H. reinwardtii*. All show the typical "*reinwardtii*" type of stomata. a: *H. r.* forma *kaffirdriftensis*, loc. 88–945 (diploid). b: *H. r.* aff. forma *chalumnensis* from Wesley (diploid). The conspicuous particles are wax. c: *H. r.* forma *kaffirdriftensis*, loc. 89–955 (diploid). d: *H. r.* forma *kaffirdriftensis*, loc. 89–958 (triploid). Cells are larger than those of the diploid from the same locality in Fig. 6c. All × c. 300.

Loc. 72: *H. coarctata* subsp. *adelaidensis*, 4x (Fig. 11a); *Epidermal cells* subconical, with rounded central papilla equivalent to about $\frac{1}{4}$ of width of cell; sculpturing composed of micropapillae showing some tendency towards aggregation into irregular ridges radiating from central papilla. Stomata with very strongly overarching lobes, outer pore small, with rectangular outline.

Loc. 81: *H. reinwardtii* var. *brevicula*, 2x; *Epidermal cells* with outer walls forming low domes; sculpturing of micropapillae showing slight tendency to random aggregation. *Stomata* with slightly incurved lobes; typical for *H. reinwardtii*, with wide rectangular outer pore.

Loc. 82: *H. reinwardtii* var. *brevicula*, 2x; *Epidermal cells* low conical, with small central papilla equivalent to about $\frac{1}{6}$ of width of cell; sculpturing composed of narrow, irregular ridges radiating from centre of cell, and occasional micropapillae. *Stomata* as for loc. 81.

Loc. 83: *H. reinwardtii* var. *huntsdriftensis*, 2x (Fig. 5c); *Epidermal cells* low conical, with or without small central papilla equivalent to about $\frac{1}{5}$ of width of cell; sculpturing composed of small, poorly defined micropapillae showing a tendency to random aggregation. *Stomata* of *H. reinwardtii* type, with wide rectangular pore, but lobes slightly overarching.

Loc. 85: *H. coarctata*; 4x; *Epidermal cells* more or less flat (Specimen no. 85–890) or strongly domed (85–900); sculpturing composed of micropapillae showing some aggregation. *Stomata* of *H. coarctata* type, with thick, overarching lobes and small rectangular outer pore, but outer wall of flanking subsidiary cells adjacent to guard cells only moderately inward-sloping.

Loc. 86: *H. coarctata,* 4x; As 85–900, but stomata with more strongly overarching lobes, and smaller outer pore.

Loc. 88: *H. reinwardtii* f. *kaffirdriftensis*, 2x (Fig. 6a); *Epidermal cells* strongly domed, some tending to conical with small central papilla equivalent to about $\frac{1}{5}$ of width of cell; sculpturing composed of micropapillae showing a little, random aggregation. *Stomata* of typical *H. reinwardtii* form but prominent since the subsidiary cells, apart from rim of pore, are lower and have micropapillae which are larger than those of other epidermal cells.

Loc. 89: *H. reinwardtii* f. *kaffirdriftensis*, 89–955, 2x (Fig. 6c); *Epidermal cells* and *stomata* as for loc. 88.

Loc. 89: *H. reinwardtii* f. *kaffirdriftensis*, 89–958, 3x (Fig. 6d); *Epidermal cells* larger and more strongly domed than in diploids, and *stomata* more prominent.

Loc. 90: *H. reinwardtii* f. *zebrina*, 2x; *Epidermal cells* slightly domed, sculpturing composed of micropapillae showing extensive random aggregation. *Stomata* of *H. reinwardtii* type but with more strongly overarching lobes than normal.

Loc. 91: *H. reinwardtii/coarctata* intermediate, 4x; A very varied population; the

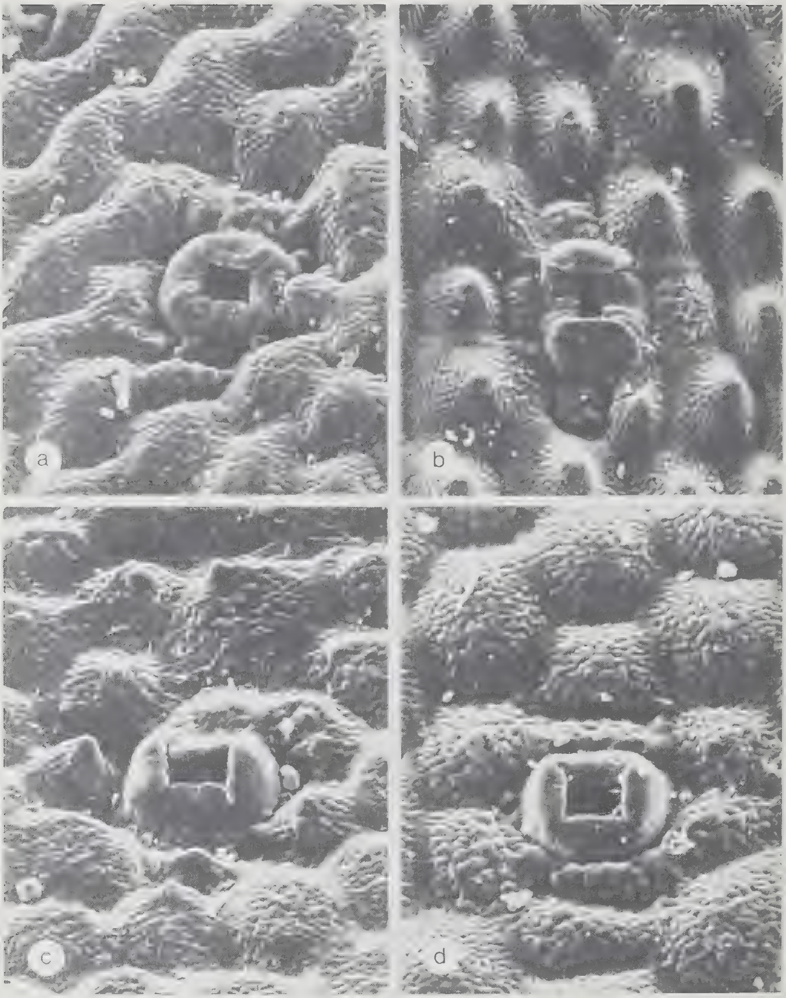

FIG. 7.
Leaf epidermis of *Haworthia* species, all with stoma types characteristic of *H. coarctata*. a: *H. coarctata*, loc. 71–687. b: *H. reinwardtii—H. coarctata* intermediate, loc. 91–1018. This shows an unusual feature, the lateral lobes of the stoma overarched by those of the poles. c: *H. coarctata*, loc. 92–1051. d: *H. coarctata*, loc. 93–1060. All tetraploids; all × c. 300.

four samples described here represent two plants tending towards the anatomy of *H. reinwardtii* and two towards *H. coarctata*.

91–1013; *Epidermal cells* slightly domed; sculpturing composed of micropapillae with some aggregation. *Stomata* of *H. reinwardtii* type but with very thick lobes.

91–1017; As 91–1013 but *epidermal cells* more strongly domed.

91–1010; *Epidermal cells* strongly domed; sculpturing composed of micropapillae with some aggregation. *Stomata* of *H. coarctata* type.

91–1018 (Fig. 7b); *Epidermal cells* with wide, sculpture-free bands above anticlinal walls; central area slightly domed to sub-conical; sculpturing of fine micropapillae in most cells aggregating at centre to form small papilla c. $\frac{1}{5}$ width of cell. *Stomata* of *H. coarctata* type but with lateral lobes slightly concave and overarched by end lobes. This unusual feature might be connected with the existence of a chromosome deletion in this plant (Fig. 4o).

Loc. 92: *H. coarctata*, 4x (Fig. 7c); *Epidermal cells* slightly domed, the dome frequently flat-topped, occasionally developed into a conical papilla; sculpturing composed of moderately coarse micropapillation with some aggregation. *Stomata* of *H. coarctata* type.

Loc. 93: *H. coarctata*, 4x (Fig. 7d); *Epidermal cells* moderately domed, position of anticlinal walls indicated by bands lacking prominent sculpturing; remaining wall area with coarse micropapillae showing some aggregation.

Loc. 95: *H. coarctata* subsp. *coarctata* var. *tenuis*, 3x (Figs 5f, 8a); Two specimens described here to show variability.

95–1075; *Epidermal cells* conical with central papilla equivalent to about $\frac{1}{4}$ width of cell; sculpturing of micropapillae, coarser on papillae than on remainder of cell, tending to aggregate into ridges radiating from centre of cell. *Stomata* with thick overarching lobes, as in *H. coarctata*, but internally more like *H. reinwardtii*. The low power photograph in Fig. 8a shows a tubercle and regular distribution of stomata.

95–1080 (Fig. 8b); *Epidermal cells* domed, occasionally subconical; sculpturing consisting of moderately coarse micropapillae showing a tendency to random aggregation; some aggregation into small papillae at apex of cones equal to c. $\frac{1}{6}$ of cell width; anticlinal wall positions defined by strips with very low relief sculpturing; *stomata* tending to *H. reinwardtii* type.

Loc. 96: *H. coarctata* subsp. *coarctata* var. *tenuis*, 2x (Figs 5e, 8c, 8d); Most *epidermal cells* low domed or sub-conical, with small central papilla about $\frac{1}{6}$ of cell width; sculpturing composed of coarse micropapillae the largest fusing to form the central papilla, others tending to aggregate and form ridges radiating from cell centre; *stomata* typical of *H. reinwardtii*, but inwardly sloping areas of flanking subsidiary cells slightly undulating and ribbed.

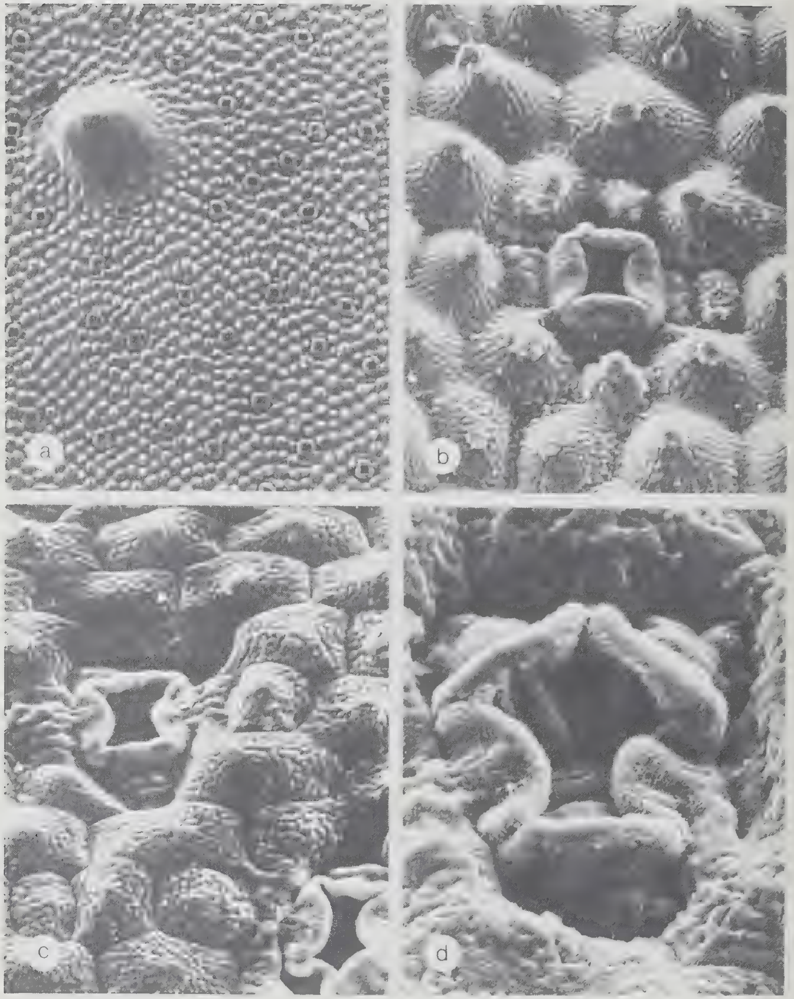

FIG. 8.
Leaf epidermis of *H. coarctata* subsp. *coarctata* var. *tenuis*. a: loc. 95–1075 (triploid). Low power photograph (× c. 40) showing a single leaf tubercle and demonstrating the uniformity of epidermal sculpturing and stoma shape over a large area. b: loc. 95–1080 (triploid), × c. 300. c: loc. 96–1089 (diploid) × c. 300. d: as c, a stoma at higher magnification showing irregular stoma lobes with ridging, × c. 660.

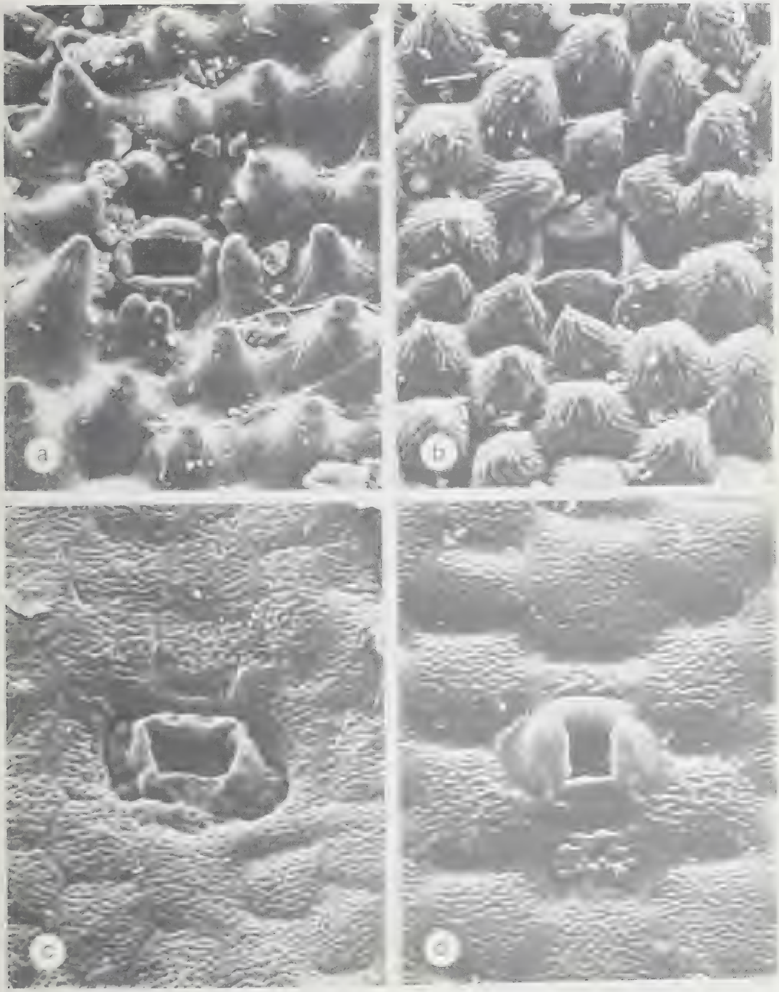

FIG. 9.
Leaf epidermis of *H. coarctata*. a. b. c: three examples of the very variable diploid population at loc. 97. a: 97–1104. b: 97–1112. c: 97–1107. d: *H. coarctata* subsp. *coarctata* var. *greenii*, loc. 103–1183 (tetraploid). All × c. 300.

Loc. 97: *H. coarctata* variable, 2x (Figs 9a, b, c); No two specimens alike. Two major trends observed; the majority with very well-developed papillae arising from conical epidermal cells; others with very slightly domed epidermal cell walls. Two examples of the former type and one of the latter are described.

97–1104 (Fig. 9a); *Epidermal cells* extended into tall cones, each terminated by a small papilla equivalent to $\frac{1}{5}$ of cell width; cones adjacent to subsidiary cells curving towards stomata. Sculpturing consisting of medium-sized micro-papillae with some aggregation to form ridges radiating from cell centre (sculpturing obscured by rather thick wax layers); *stomata* most like *H. reinwardtii* type.

97–1112 (Fig. 9b); *Epidermal cells* extended into medium-sized cones each with a poorly defined central "papilla" formed by aggregation of coarse micropapillae; flanks of cone with sculpturing of coarse micropapillae aggregating into irregular ridges radiating from cell centre; cones adjacent to subsidiary cells curving towards stomata. *Stomata* of *H. reinwardtii* type but with polar lobes overarching.

97–1107 (Fig. 9c); *Epidermal cells* more or less flat or low-domed; outlines of cells clearly defined by double lines devoid of sculpturing; outer wall sculpturing composed of micropapillae finer than those of 97–1112, showing some random aggregation; *stomata* of *H. reinwardtii* type.

Loc. 98: *H. coarctata* 4x; *Epidermal cells* low domed; sculpturing composed of moderately pronounced micropapillae showing some aggregation into short irregular ridges arranged across width of the cells; *stomata* of *H. coarctata* type; guard cells not as deeply sunken as in most other specimens.

Loc. 100: *H. coarctata* subsp. *adelaidensis* 2x (Figs 10a, b, c); Specimens from this locality were variable. Epidermal cells low domed in 100–1153 (Fig. 10a), produced into small irregular cones in 100–1149 (Fig. 10b) or well-developed cones with small central papilla in 100–1143 (Fig. 10c); sculpturing of medium-sized (100–1143 and 1153) or coarse micropapillae (100–1149), with a considerable tendency to irregular aggregation in 100–1149 or to radiating ridges in 100–1143. Micropapillation not noticeably larger on subsidiary cells in 100–1143 or 100–1149 contrary to that on almost every other plant in this study; *stomata* in all samples of *H. coarctata* type.

Loc. 101: *H. coarctata* subsp. *adelaidensis* 2x (Fig. 10d); Specimens from this locality variable; 101–1161 similar to 100–1153; 101–1163 (Fig. 10d) similar to 101–1161 except for each epidermal cell bearing a small central papilla equal in diameter to about $\frac{1}{6}$ of cell width. 101–1159 resembling 100–1143 except that cells smaller.

Loc. 102: *H. coarctata* subsp. *coarctata* var. *greenii* 4x; *Epidermal cells* moderately domed; sculpturing of fine to medium micropapillae with a tendency to random aggregation; *stomata* of *H. coarctata* type.

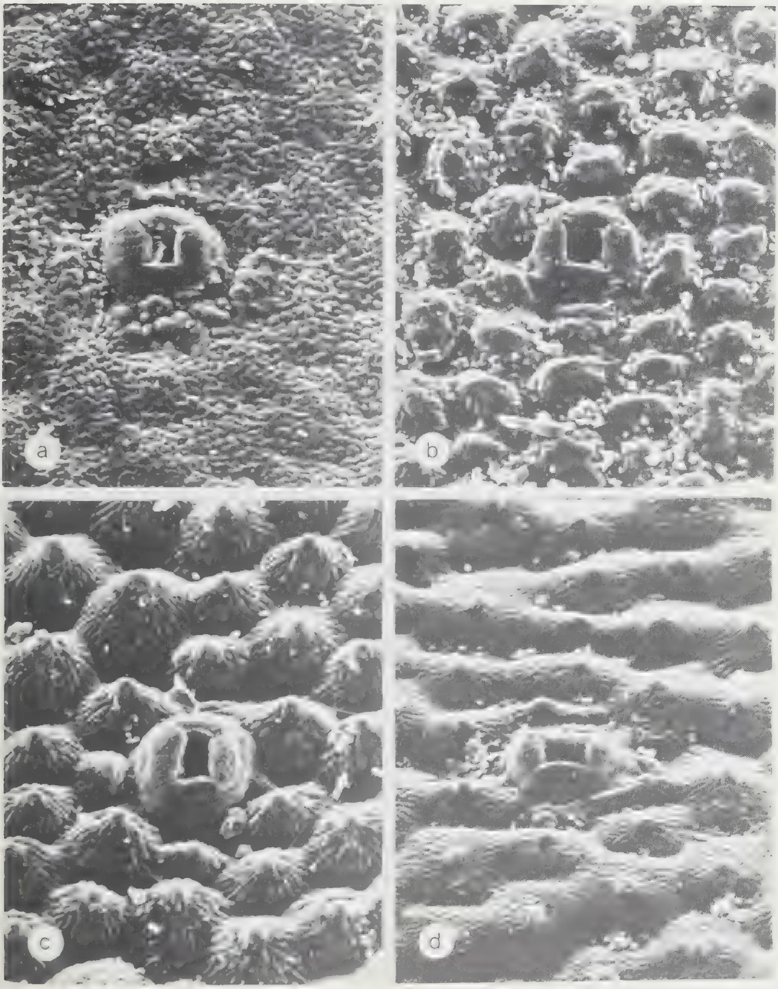

FIG. 10.
Leaf epidermis of *H. coarctata* subsp. *adelaidensis*. a, b, c: three examples of the variable diploid population at loc. 100. a: 100–1153, b: 100–1149. c: 100–1143. d: loc. 101–1163 (diploid). All × c. 300.

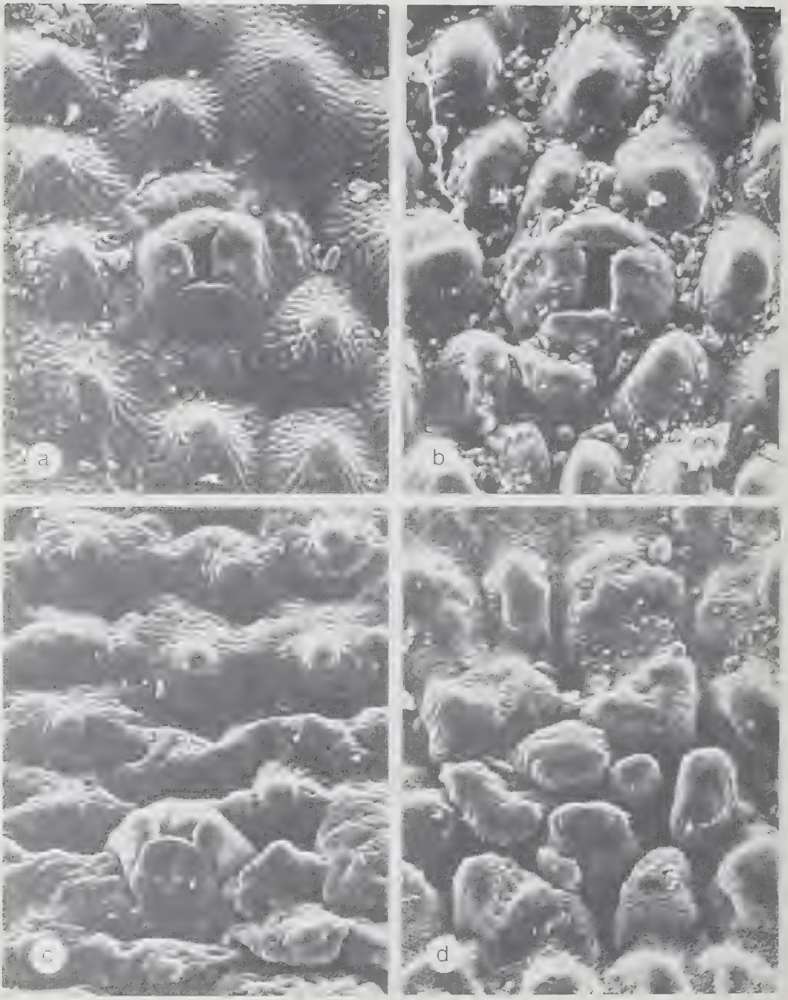

FIG. 11.
Leaf epidermis of *Haworthia* species. a: *H. coarctata* subsp. *adelaidensis*, loc. 72–707 (tetraploid) with larger cells than in diploid plants of the same subspecies (Figure 10). b: *H. glauca* var. *herrei* forma *armstrongii*, loc. 112–1277 (pentaploid). c: *H. coarctata*, hexaploid from Rooidrift. d: *H. glauca*, loc. 117–1321 (hexaploid). The suprastomatal lobes are very thick, making it difficult to determine the position of the stomata. All × c. 300.

Loc. 103: *H. coarctata* subsp. *coarctata* var. *greenii* 4x; Most specimens as for loc. 102 (Fig. 9d), but one specimen with concave flanking lobes of the stoma.

Loc. 104: *H. coarctata* subsp. *coarctata* var. *greenii* 4x; 104–1196, as for loc. 102 but *epidermal cells* not so domed; 104–1197 as for loc. 102 but most *epidermal cells* with a small central papilla equivalent in diameter to $\frac{1}{5}$–$\frac{1}{6}$ of cell width. Occasional anomalous 5-lobed *stomata* present in 104–1197.

Bayer Loc. 91: Wesley, *H. reinwardtii* aff. f. *chalumnensis* 2x (Fig. 6b); *Epidermal cells* subconical, most with small central papilla with diameter equivalent to $\frac{1}{6}$ of cell width; sculpturing of moderately coarse micropapillae showing some aggregation; *stomata* prominent, of *H. reinwardtii* type.

Bayer s.n. Rooidrift, *H. coarctata* 6x; *Epidermal cells* conical with somewhat overarching anticlinal walls; most with large terminal papilla equal to $\frac{1}{4}$–$\frac{1}{3}$ of width of cell; sculpturing of low relief coarse micropapillae obscured by wax covering; *stomata* intermediate between *H. coarctata* and *H. reinwardtii* types with somewhat irregular lobes.

Loc. 112: *H. glauca* var. *herrei* f. *armstrongii* 5x (Figs 5d, 11b); *Epidermal cells* conical, most with central papilla equivalent in diameter to $\frac{1}{2}$–$\frac{1}{4}$ of width of cell; sculpturing of coarse, low relief micropapillae; *stomata* of *H. coarctata* type but with very thick overarching lobes bordering a small outer aperture.

Loc. 117: *H. glauca* 6x (Figs 5g, 11d); *Epidermal cells* with irregular strongly domed outer walls, each like a stubby papilla; those next to subsidiary cells curving towards stoma; sculpturing not observed; part of surface obscured by fine upright wax flakes; *stomata* with four distinct very much enlarged lobes only slightly smaller than the papillae of the adjacent cells; lobes almost closing suprastomatal cavity.

Ploidy level and cell size

The number of cells per unit leaf area was scored in 102 plants of all levels of ploidy from 2x to 6x. These figures, together with a conversion into relative mean cell surface area are given in Table 2.

DISCUSSION

The significance of epidermal features and polyploidy in relationships between populations

Within the *Aloineae*, the details of stomatal structure and cell wall/cuticular sculpturing have been shown to be of diagnostic, and in some instances, of taxonomic significance (Cutler, 1979). Confusion has arisen frequently regarding the identification of plants in the section *Coarctatae* of the genus *Haworthia*. In particular, *H. reinwardtii* and *H. coarctata* together with their varieties and forms are easily confused. The problems have been made worse since the plants are widely cultivated: many varieties and forms have been described from culti-

Table 2.
Ploidy level, cells per unit area and relative areas of individual cells.

Ploidy level	2x	3x	4x	5x	6x
No. of plants scored	40	7	40	6	9
Mean no. of cells per unit area	44	36	35	25	26
Relative areas of individual cells	1	1,22	1,26	1,76	1,69

vated material. The majority of taxa in this group are interfertile and undoubtedly many hybrids have arisen which are not recognised as such. Intensive selection by collectors has also provided plants for which new names have been given which are of no taxonomic significance (e.g. the many works of Resende, quoted by Bayer, 1976:177).

It was essential to return to localities in the wild to find suitable material so that a rational approach could be made to the classification of this group. Bayer (1973, 1976) has recently done this. As a result of his studies he eliminated or changed the rank of a large number of taxa. He relied heavily on morphology and distribution. The flowers provide little of significance whereby species can be identified, being extremely uniform throughout the group.

During the period that Bayer was studying these plants, we were working on the Kew collections. We recognised the need for wild-source material and were able to collaborate with him in making additional collections from sites which he showed us. We were then able to study the anatomy and cytology of the plants, mainly to see whether there was anything of taxonomic significance that could be derived from these new approaches.

The chromosome results revealed that both *H. reinwardtii* s.l. and *H. coarctata* s.l. contained diploid, triploid and tetraploid plants (also hexaploid in *H. coarctata*). This, then, provided little help with classification. The chromosome interchanges, although of considerable interest as far as chromosome mechanics are concerned (see below), similarly provide no taxonomic information because no two populations contained the same interchange. This is unlike the situation in *H. reinwardtii* f. *chalumnensis* in which inter-population interchange distribution was a valuable aid to determining evolutionary trends (Brandham, 1974).

The anatomical data on their own gave some help towards an understanding of the problem, but contained apparent anomalies. On the whole, plants called *H. coarctata* by Bayer showed one type of stoma, whereas those called *H. reinwardtii* had another distinct type. There were exceptions, where plants with the general morphology of *H. coarctata* had the stomatal configuration of *H. reinwardtii*, e.g. *H. coarctata* subsp. *coarctata* var. *tenuis* at Loc. 96 (Figs 8c, d) and *H. coarctata* from Loc. 97 (Fig. 9c). Bayer included some populations of plants in *H. coarctata* because of their geographical distribution, even though they might be mistaken for *H. reinwardtii*. These were found to have the *H. coarctata* type of stoma, e.g. *H. coarctata* var. *adelaidensis* at Locs 100 and 101 (Figs 10a, b, c, d). It was necessary to combine the cytological and anatomical approaches to make progress in clarifying this involved biological problem.

We have been able to describe here all of the cytological data, but space has restricted us to selecting only a small proportion of the anatomical information for presentation. Great care was taken to select representative plants and where variability existed in populations, to demonstrate the range.

Photographs of the leaf surfaces of the entire collection were laid out in a geographical arrangement, together with details of chromosome numbers. An attempt was made to elucidate affinities, and our results are based on those observations.

The genetic variability within this complex was reflected in leaf surface sculpturing, as we would have expected from previous studies (Brandham and Cutler, 1978).

Interchange and deletions do not on the whole seem to be detectable as consistent variations in the anatomy of these plants, as was the case in *H. reinwardtii* f. *chalumnensis* (Cutler, 1978a), but in the present study much smaller sample numbers were examined from individual populations. It is possible that there might be a detectable relationship if larger numbers could be studied. However, the abnormal stomatal structure in the *H. reinwardtii/coarctata* intermediate, no. 91–1018 (Fig. 7b) could be associated with the chromosome deletion that it carries (Fig. 4o).

Inter-population affinities

(a) *Diploids*

Four main geographically distinct groups of diploids were found (Fig. 12). It seems probable that they may be relicts of a larger population. The extensive variability which they display is not surprising in such relict groups.

The three western groups share some of the characteristics associated with *H. coarctata*, but also exhibit close links with *H. reinwardtii* to the east. The northernmost of these groups (Locs 100, 101) show morphological similarity to *H. reinwardtii* as defined by Bayer, but have the stomatal type which in this paper we associate with *H. coarctata*. Bayer has treated this group as *H. coarctata* on the basis of its distribution, since it is close to the type population of *H. coarctata* subsp. *adelaidensis* (Loc. 72).

The west-central group of diploids (Loc. 97) shows considerable variation in both morphology and leaf surface sculpturing. Because of their distribution, Bayer (1973, 1976) has included these plants with *H. coarctata*, but it can be seen from Figs 9a, b, c that they have a stomatal type reminiscent of *H. reinwardtii*.

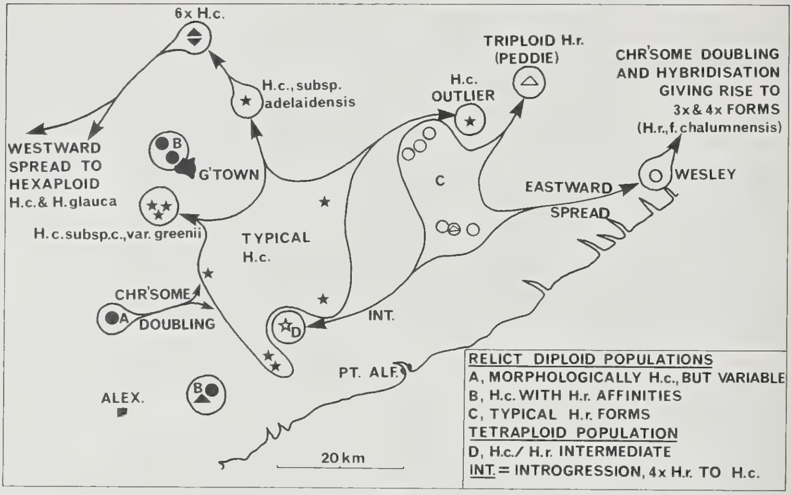
6x H.c.
TRIPLOID H.r. (PEDDIE)
CHR'SOME DOUBLING AND HYBRIDISATION GIVING RISE TO 3x & 4x FORMS (H.r., f. chalumnensis)
H.c., subsp. adelaidensis
H.c. OUTLIER
WESTWARD SPREAD TO HEXAPLOID H.c. & H. glauca
B
G'TOWN
C
EASTWARD SPREAD
WESLEY
H. c. subsp. c., var. greenii
TYPICAL H.c.
CHR'SOME DOUBLING
A
INT.
D
PT. ALF.
ALEX.
B
20 km
RELICT DIPLOID POPULATIONS
A, MORPHOLOGICALLY H.c., BUT VARIABLE
B, H.c. WITH H.r. AFFINITIES
C, TYPICAL H.r. FORMS
TETRAPLOID POPULATION
D, H.c./ H.r. INTERMEDIATE
INT. = INTROGRESSION, 4x H.r. TO H.c.

Fig. 12.

Map of the Grahamstown – Port Alfred area showing probable evolutionary trends in *Haworthia reinwardtii* (H. r.), *H. coarctata* (H. c.) and related forms. A full explanation is given in the text. Symbols are as in Figure 1.

Plants at the limit of their range of distribution are often genetically diverse and this seems to be the case here. Plants with the two extreme types of epidermal cell sculpturing illustrated by Figs 9a and 9c might be expected to produce hybrids with an intermediate type such as that shown in Fig. 9b. Controlled crossing experiments by Cutler and Brandham (1977) using other members of the *Aloineae* revealed that F_1 hybrids normally show a type of surface sculpturing intermediate between those of the parents.

The south-west group of diploids (Locs 95–96) consists of plants with a distinctive, slender form. It includes triploids of local origin (Loc. 95) which are not distinguishable from the diploids using characters of gross morphology for comparison. However, the diploids have stomata reflecting similarity with *H. reinwardtii* and the triploids show a stronger tendency to exhibit the *H. coarctata* form of stomata. It may be that the triploids are expressing a dosage effect for the *coarctata* stomatal type. Bayer calls these plants *H. coarctata* var. *tenuis*.

The major group of diploids is centred on the Fish River, as exemplified by Localities 81, 82, 83, 88, 89 and 90 in this study. Local triploids also occur at Locality 89. These plants have been grouped together by Bayer as *H. reinwardtii*. They do show some variation in both anatomy and morphology which previously has been recognised in the described varieties *brevicula* and *huntsdriften-*

sis and the forms *kaffirdriftensis, zebrina* and *olivacea*. However, there are no anatomical variations which correlate with any of these varieties or forms. All exhibit the *H. reinwardtii* type of stoma.

Among all diploid groups, then, there are hints, either in morphology or anatomy, of a common ancestry. Separation of the western populations as *H. coarctata s.l.* seems to be rather arbitrary.

(b) *Tetraploids*

There are two major groups of tetraploids. One, which is described as 'typical *H. coarctata*' by Bayer (1976), occupies a broad zone between Grahamstown and Port Alfred (Localities 85, 86, 92, 93 and 98). The other, *H. reinwardtii* f. *chalumnensis* (Smith) Bayer, occurs much further east of the main diploid *H. reinwardtii* complex, at and around Chalumna. This second group was the subject of earlier papers (Brandham 1974; Cutler, 1978a). In the first of these two papers the conclusion was reached that evolution had taken place from diploids in the west to triploids and tetraploids in the east, via the diploids growing at Wesley, and our find of more diploid *H. reinwardtii* in the Fish River valley confirms this conclusion.

The western group of tetraploids is of principal concern to us in this paper. Members of this group show closest morphological affinities with the diploids to their west at Locality 97 near Salem. We suggest, therefore, that they could have arisen by chromosome doubling in diploids from this area.

This western group of tetraploids appears to be expanding territorially in several directions. There is an eastern outlier at Locality 71, Huntsdrift, a western outlier with some morphological differentiation (described as *H. coarctata* subsp. *coarctata* var. *greenii* (Baker) Bayer) at Howiesonspoort, Localities 102, 103 and 104, and a northern form at Locality 72, Queens Road, NE of Grahamstown, which is slightly smaller, and is called *H. coarctata* subsp. *adelaidensis (von* Poelln.) Bayer. All of these plants show the typical '*H. coarctata*' type of stoma, with some slight variations in epidermal cell cuticular sculpturing.

A particularly interesting population of tetraploids is found at Locality 91. Bayer (pers. comm.) described these plants as intermediate between *H. reinwardtii* and *H. coarctata*. The population is cytologically very unstable, with six mutant karyotypes (Table 1) in addition to plants with apparently normal chromosomes. The stomata have concave lateral lobes, a feature which is unusual in *Haworthia* but which has been observed in other members of the *Aloineae* by the present authors (unpublished).

There is a distinct possibility that this is a hybrid population derived from tetraploid *H. coarctata* nearby with gene introgression from undiscovered or extinct tetraploids of the normally diploid *H. reinwardtii* growing to the east, near the Fish River.

(c) *Pentaploids*

There appears to be only one pentaploid, called *H. glauca* var. *herrei* f. *armstrongii* (von Poelln.) Bayer. It shows clear affinities with tetraploid *H. coarctata* in its leaf surface structure. It cannot be conspecific with *H. glauca* on either cytological or anatomical evidence, since *H. glauca* is hexaploid and has a most unusual leaf surface (Fig. 11d). We therefore propose reinstatement of the name *H. armstrongii* von Poelln. for this taxon.

There is controversy over whether a single clone of probably hybrid origin should be given specific rank. Here it seems reasonable since the plants are morphologically distinct and are genetically isolated from crossing with sympatric species of *Haworthia*, since they are pentaploid and totally sterile. A precedent for this decision is *H. browniana* von Poelln. which has also been shown to be a single clone occurring in one locality (Brandham and Johnson, 1977a).

(d) *Hexaploids*

The hexaploid specimens of *H. coarctata* from Rooidrift to the north of the main distribution could have arisen from the western tetraploid group by fusion of a normal and an unreduced gamete. Other hexaploid *H. coarctata* occupying habitats on high ground to the west of the Rooidrift population are probably all closely related and link with the hexaploid *H. glauca* at Locality 117. However, *H. glauca* (Figs 5g, 11d) is anatomically distinct from the other hexaploids, which have a type of stoma intermediate between those of *H. coarctata* and *H. reinwardtii*, with slightly irregular lobes.

One published record (Snoad, 1951) states that *H. glauca* is tetraploid, but since he worked mainly on cultivated material of unknown wild origin the identity of his specimen could have been in doubt.

Figure 12 summarises the inter-population affinities that we have described above and indicates the probable evolutionary trends within the group.

Ploidy level and cell size

Reference to Table 2 shows that there is not a steady increase in cell size in relation to ploidy level. Cells of triploids and tetraploids are larger than those of diploids but are similar in area to each other. Cells of pentaploids and hexaploids are still larger in area but are also similar to one another.

It has been noted that "odd number" polyploids are often disproportionately vigorous (e.g. Williams, 1964). This may well explain the results here, where triploids have cells which are as large in mean surface area as those of tetraploids, and pentaploids have cells slightly larger on average than those of hexaploids.

If we consider just the even number polyploids we find that there is a progressively larger cell surface area with increased ploidy level. Cell volumes cannot be calculated from these data. The thickness of epidermal cells in relation to cell height and width is variable between specimens and each one would need to be measured individually if such calculations were required.

The significance and distribution of the chromosome interchanges

It has been stated above that a certain amount of morphological variation was encountered in several populations of the species studied. We could find no correlation between these variants and whether or not they were interchange heterozygotes. Thus no position effect of the alteration of the gene linkage groups associated with chromosome interchange could be detected, unlike that which was found earlier in *H. reinwardtii* f. *chalumnensis* (Cutler, 1978a). However, the interchanges were found only in the heterozygous state in the diploids and as simplex heterozygotes in the tetraploids, so it must remain uncertain whether a position effect of the chromosome rearrangements would be apparent in interchange homozygotes.

The occurrence of interchanges in the *Aloineae* is of considerable interest as a source of potential karyotype alteration and evolution in this group, which is otherwise very stable in its gross chromosome morphology. Curiously, the distribution of interchange hybridity in the tribe is markedly non-random. We have examined the chromosomes of many thousands of individuals encompassing the entire tribe and have observed interchanges to occur extremely sporadically in the genera *Aloe* and *Gasteria*, which are close relatives of *Haworthia*, (Brandham 1969, 1973, 1976; Brandham and Johnson, 1977a); the incidence of interchange hybridity being in the order of 1 %.

On the other hand this study has shown that the interchange frequency is much higher in *Haworthia* section *Coarctatae* than in the rest of the tribe or even the rest of the genus, interchange hybridity having been found in 29 of the 282 plants studied here, an overall frequency of 10,3 % (Table1). Within the *Coarctatae* this very high figure is greatly exceeded in *H. reinwardtii* f. *chalumnensis*, in which of 145 individuals that were studied 113 contained interchanges, a frequency of 77,9 % (Brandham, 1974).

The reason why interchanges should be so common in the section *Coarctatae* of *Haworthia* and relatively rare in the rest of the *Aloineae* remains unknown. Within the tribe as a whole polyploidy is also largely confined to the *Coarctatae*, but any suspected correlation between polyploidy and high interchange frequency must now be discounted in view of the fact that polyploid *Aloe* species and polyploid *Haworthia* species outside the *Coarctatae* were found to have a low frequency of interchange hybridity in common with the remainder of the tribe (Brandham, 1976; Brandham and Johnson, 1977a).

The distribution of interchange hybridity is thus grossly non-random among the individual plants of the *Aloineae* sampled to date. Within the karyotype some earlier reports have indicated that the break points associated with inter-

Table 3.

Positions of 52 chromosome breaks involved in 26 interchanges in *Haworthia* sect. *Coarctatae*. Data from present study and from Brandham (1974); those for the short chromosomes S_1 S_2 S_3 are derived from the total short chromosome breaks divided into three equal proportions (see text).

Chromosome -no.	No. of breaks at different positions on chromosomes			
	Short arm	Centromere	Long arm (P = proximal, D = distal)	Total breaks
L_1	2	3	1P, 2D	8
L_2	1	0	3P, 5D	9
L_3	0	3	3P, 4D	10
L_4	2	1	4P, 3D	10
S_1	0,66	2,33	2	5
S_2	0,66	2,33	2	5
S_3	0,66	2,33	2	5

change are also non-randomly distributed within and between the individual chromosomes. For instance, Riley and Majumdar (1968) summarised their previous work on interchanges in *Haworthia*, surveying six straighforward exchanges and one other in which breaks had occurred in three chromosomes. They found that the positions of the break points in the chromosomes involved in the exchanges were not randomly distributed. Of their total of 15 breaks no fewer than 13 were at the centromeres and only two were elsewhere. Furthermore, the L_1 chromosome was involved in six of the seven interchanges; and this too is clearly not a random occurrence.

Observations on 12 interchanges in *H. reinwardtii* f. *chalumnensis* by Brandham (1974) were added to those of Riley and Majumdar and tended to confirm this non-random distribution of break points, but when the f. *chalumnensis* results are added to the 14 interchanges described in the present study (those of Riley and Majumdar being excluded) a different picture emerges. In these two studies the 26 interchanges which have been analysed and illustrated provide a total of 52 chromosome breaks, which is a sample large enough to form the basis for a more meaningful survey of break point distribution, restricted as it is to only two very closely related species within the genus.

The positions of these breaks are given in Table 3. In this table the total of 15 short chromosome breaks has been arbitrarily divided among the three short chromosome sets, the S_1, S_2 and S_3. This was necessary because it will be recalled that no attempt was made to separate these extremely similar chromosomes into three sets of homologues. The table shows that in this sample of interchanges the breaks are largely random in distribution, a finding which is contrary to that of Riley and Majumdar (1968). There is no tendency for the L_1 to be involved any more frequently than the other long chromosomes. In fact it is apparent that breaks occur in all four long sets with approximately equal frequency. There is, however, a slight tendency for more breaks to occur in the short chromosomes than would be expected on the assumption that break frequency is proportional to chromosome length. The frequency in the short chromosomes can be seen from Table 3 to be half that in the long chromosomes, whereas the illustrations in this article (e.g. Fig. 4) show that the actual length of the short chromosomes is very much less than half that of the long ones.

Within chromosomes the break points are also distributed almost at random with only a very slight tendency towards preferential breakage through the centromere, which is a recognised "weak point" (Brandham, 1974). Assuming complete randomness, the frequency of breaks through the centromere should drop almost to zero. Table 3 shows that although the frequency of such breaks is lower than that found by Riley and Majumdar (1968) they are nonetheless a significant proportion of the total number of breaks.

Several of the interchanges in *Haworthia* are of the centric fusion type, with breaks through or near the centromeres of two acrocentrics followed by reunion to give a large metacentric derived from the long arms and a short metacentric or submetacentric from the short arms. Examples are interchange A, B and E in *H. reinwardtii* f. *chalumnensis* (Brandham, 1974), and 3, 5 and 7 in this study.

This type of chromosome interchange is well known under the term Robertsonian fusion, and in many organisms the shorter interchange chromosome is lost following such an event, the interpretation of which is that the organism can tolerate the loss of the genes situated on the short arms (if any). With the establishment of homozygosity for this type of Robertsonian fusion there is a net loss of two chromosomes from the complement (in fact only two centromeres and two pairs of short arms are lost) and a new stable karyotype is established (Jones, 1974). In all records of Robertsonian fusion in the *Aloineae* published to date by Riley and his co-workers and by ourselves (Brandham, 1973, 1974, 1976; Brandham and Johnson, 1977a) the short interchange chromosome is never lost and there is no net lowering of the chromosome number. It is thus apparent that the short arms of the acrocentric chromosomes of *Haworthia* and probably of the rest of the *Aloineae* possess genes without which the plants cannot survive.

One problem remains to which no satisfactory answer can yet be put forward. Although interchanges are by now known to occur throughout the *Aloineae* and are even relatively common in the section *Coarctatae* of *Haworthia* no instance has yet been found of a naturally-occurring homozygote. As a result of a Robertsonian interchange involving non-homologous long acrocentric chromosomes in which the arms are rearranged following breakage to produce a very long metacentric and a short metacentric, a diploid homozygote would have one pair of very long metacentrics, two pairs of normal long acrocentrics, one pair of short metacentrics and three pairs of normal short acrocentrics. Such a homozygote would be expected to have regular meiosis, forming seven bivalents, and it would be fully fertile. The karyotype would then constitute a major departure from the standard *Aloineae* karyotype and would form the foundation of further evolutionary departures since it would be to some extent isolated genetically from the non-interchange homozygotes.

The reason why these homozygotes have not occurred, or if they do occur, the reason why they do not persist is not known. Certainly interchange heterozygotes in the *Aloineae* are capable of reproducing sexually in the wild, as has been shown earlier, both in *Aloe* (Brandham and Johnson, 1977a), and in *Haworthia reinwardtii* f. *chalumnensis* (Brandham, 1974). This observation is confirmed by the present study, which has demonstrated that sexually-produced rearrangements of different interchanges occur at Locality 91. Those involved are interchange 1A or one of its altered chromosomes ("Deletion 2"), which are found either alone or associated with interchanges 3 or 4 in different plants (Table 1).

It is therefore theoretically possible for interchange homozygotes to occur in the wild in the seed progeny of heterozygotes in the second or further generations following the initial mutation (allowing for the fact that most *Aloineae* are self-incompatible), but it is evident that since they have not been found they must be selected against if formed. The basic karyotype of four long and three short acrocentric chromosomes in the *Aloineae* is therefore maintained.

White (1963) introduced the term "karyotype orthoselection" as a mechanism by which the basic karyotype of a taxon or series of related taxa remains unchanged despite the sporadic occurrence of various chromosomal changes in the heterozygous condition. It was considered that the particular karyotype typical of the taxon is the optimal one. All deviant karyotypes are thus subjected to adverse selection pressures and are eliminated. Even though structural chromosome changes are now known to occur at quite high frequencies in *Haworthia* section *Coarctatae*, and at lower but still significant frequencies in the rest of the *Aloineae* they have yet to be found as homozygotes. The stable bimodal karyotype which is found in every one of the hundreds of species comprising the *Aloineae* tribe may therefore be considered as one of the strongest known examples of karyotype orthoselection.

ACKNOWLEDGMENTS

We thank Mr. M. B. Bayer of the Karoo Botanic Gardens, Worcester for showing us the sites of most of the plants used in this study. Mrs. R. Gale and Mr. E. Borez gave technical assistance with the electron microscopy, and Miss M. Johnson with the cytology.

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